UCRL-JC-124552 ABS

Concurrent Session-Materials Preparation

An Automated Filter Hybridization Protocol and Longer Read Lengths Improve the Output and Efficiency of Shotgun Cloning.

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The Livermore Genome Center is utilizing a shotgun based approach to sequence chromosome 19 and targeted gene regions of interest. Many of the preparative steps have been automated e.g. sample tracking, plaque/colony picking, template preparation and sequencing reactions. In addition, we have improved our sequence output and efficiency by optimizing a number of factors that affect sequence quality such as increasing the running buffer concentration, using a modified acrylamide matrix, changing the sequencing polymerase and concentration, shifting to energy transfer dye primers, modifying the cycling parameters and decreasing the electrophoresis field strength. These modifications have increased our confident read length from an average of 450 bases to over 700 bases. Signal strength now extends well over 1200 bases and the signal in between 800 and 1000 bases having the potential to be manually edited. We have also developed a filter hybridization protocol that is largely automated and able to screen out nonproductive clones in a fast, easy, and cost effective manner. M13 clones are robotically stamped onto nylon filters, hybridized to the cosmid and vector and scanned into a PhosphorImager. The resulting image files are analyzed to generate a re-array command file used by a robot to inoculate the selected clones for template isolation and sequencing. We have found that between 80-90% of the clones containing no insert or with a cosmid vector insert were effectively eliminated from the re-arrayed population. When the re-arrayed vs. randomly chosen clones were assembled separately, the contigs generated and the depth of coverage appeared comparable, showing that the library content was equivalently represented by both sets of clones. Additionally, the library filters represent a resource from which we can probe for clones to be used for gap closure when necessary. This work was performed by Lawrence Livermore National Laboratory under the auspices of the US Department of Energy, Contract No. W-7405-Eng-48.

8th Int'l Genome Sequencing and Analysis Conference
Hilton Head Island, SC, October 5-8, 1996